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ABSTRACT OF THE DISSERTATION

Luciferases of Luminous Beetles:
Evolution, Color Variation, and Applications

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by
Keith V. Wood

Doctor of Philosophy in Chemistry
University of California, San Diego, 1989
Professor William D. McElroy, Chairman

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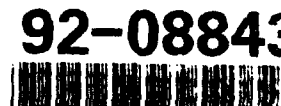
The biochemical basis of beetle luminescence is unrelated evolutionarily to other bioluminescent systems that have been characterized at a molecular level, such as of marine invertebrates (e.g., *Aequorea* or *Vargula*) or bacteria. To advance the study of the beetle system, cDNA's coding several luciferases have been cloned and expressed in *Escherichia coli*. Cells expressing the cDNA clones can be induced for luminescence when the substrate, luciferin, is supplied to the growth medium. The physical and enzymatic properties of the beetle luciferases synthesized in *E. coli* were found to be essentially identical with native enzymes extracted from beetle light organs. This equivalence of enzyme derived from native and recombinant sources indicates that beetle luciferases are homomeric enzymes without substantial covalent modification of the primary translation product (e.g., proteolytic cleavages or glycosylations). The most characterized of the beetle luciferases has been that of the common North American firefly, *Photinus pyralis*. A cDNA coding this luciferase was the first to be cloned, and can be expressed in *E. coli* at roughly



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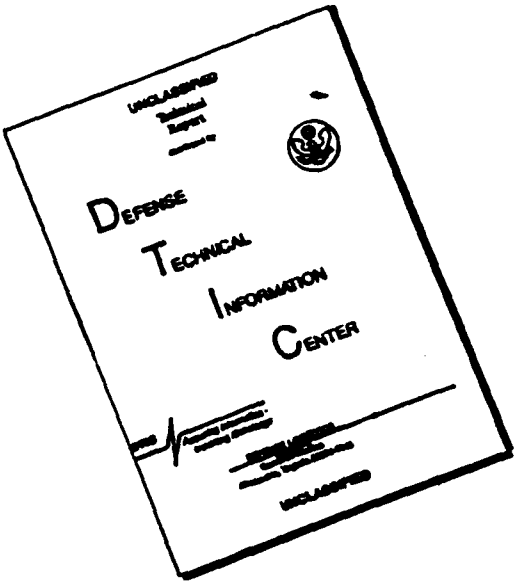

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100 mg of functional enzyme per liter of culture. Subsequently, several cDNA's coding luciferases from the ventral light organ of a tropical click beetle, *Pyrophorus plagiophthalamus*, were similarly cloned and expressed. These clones are of four types, distinguished by the colors of luminescence they elicit: green, yellow-green, yellow, and orange. Color variation among specimens is a unique property of this beetle species. The colors likely are derived from alleles that have evolved in their spectral order, from green to orange. The amino acid sequences of the click beetle luciferases are highly similar among themselves, ranging from 96 to 99% identical. The amino acids that determine the different colors are few and act independently. The sequences of click beetle luciferases compared with the firefly luciferase reveal only ±8% identity. Substantial differences are also evident in their enzymological properties. Similarity has been found between the sequences of the beetle luciferases and a plant enzyme, 4-coumarate:CoA ligase. Comparison of the enzymatic activities of these enzymes show that the evolutionary ancestors of beetle luciferases were coenzyme A ligases; the oxygenase activity was a more recent acquisition.

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